Washington State Department of Health

ELABORATIONS

News and Issues for Washington's Clinical Laboratories

Volume VIII Issue 9 October 2003

FDA Recalls Tests:

CoaguChek Prothrombin Time Test Strip
VIDAS Chlamydia Assay

CoaguChek: According to a national recall notice on the Food and Drug Administration's Web site, Roche Diagnostics is notifying users of an important recall of all CoaguChek PT test strips currently in the marketplace because of the potential for a packaging defect that will cause false results.

CoaguChek PT test strips are used by patients in the home and by professionals in medical settings to determine blood clotting time of patients taking anti-coagulants and to diagnose some disease conditions. Incorrect results may have serious or life-threatening consequences because patients might be improperly diagnosed or improperly treated. Blood thinners are used to treat patients with a potential for blood clots. For example, patients with heart valve replacement, certain types of heart disease or blood clots in their legs.

Roche has determined that some of the foil pouches in which the test strips are packaged were improperly sealed allowing moisture and air to enter the pouch. These products give false results when exposed to moisture for more than a few minutes. Home users should contact their health-care professionals for further advice and instructions.

Roche is notifying all home users and health-care professionals who use the product to inspect the foil pouch before use and to perform duplicate testing for all lots until further notice. The problem is the perforation and the "easy open" notches are not properly centered between the pouches. Users must inspect each pouch before use, not use any strips from that box if they see a defect, and run two test strips each time they test in case they fail to visually detect the defect.

Investigations reveal that only a small percentage of the strips are affected. No illnesses or injuries resulting from a pouch defect have been reported.

Letters are being sent to customers, providers, and physicians, informing them of this voluntary action. Further updates and additional information, including a photograph of the defective pouch, will be posted on

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Inside This Issue

Practice Guidelines

The following practice guidelines have been developed by the Clinical Laboratory Advisory Council. They can be accessed at the following website:
www.doh.wa.gov/lqa.htm

- Anemia
- ANA
- Bioterrorism Event Mgmt
- Bleeding Disorders
- Chlamydia
- Diabetes
- Group A Strep Pharyngitis
- Hepatitis
- HIV
- Intestinal Parasites
- Lipid Screening
- Point-of-Care Testing
- PSA
- Red Cell Transfusion
- Renal Disease
- STD
- Thyroid
- Tuberculosis
- Urinalysis
- Wellness

Bioterrorism Corner: Biodetection Devices

by Candace Bunch

In this day of potential bioterrorism, the sooner an attack can be detected the sooner a coordinated response can begin. Detection and surveillance can be used to combat a covert event. However, it is also true that accuracy is more important than instant answers.

At this time, the U.S. Department of Health and Human Services is recommending against the use of hand-held assays by first responders for the evaluation of unknown powders suspected to be anthrax or other biological agents. There are a variety of reasons. In some cases, field equipment provides an apparent (or false) "positive" result that can lead to the unnecessary quarantine, isolation or decontamination of people. When these samples are referred to a reference lab they are found to be negative through culturing and molecular methods. Reasons for false positives may include testing in the presence of caustic or harsh chemicals, other interfering substances, or inadequately trained personnel.

Hand-held assays can also yield negative results on samples that are truly positive (false negatives). The analytical sensitivity of these assays is limited by the technology. Current data indicates that a minimum of 10,000 spores are required to generate a positive signal for Anthrax. This would require a sample from a highly contaminated area and is far more than required to cause infection. In the laboratory a culture can detect a single spore of Anthrax.

Novel detection methods are currently in the testing phase. Field testing, however, has been performed on two basic types of detection methods developed in miniaturized and mobile packages: Real-time PCR technology that can provide both quantitative and highly specific recognition but is highly dependent on user capability, and fluorescent antibody technology that may be more qualitative and not as sensitive as PCR based technologies. Antibody-based reactions and their levels of detection depend heavily on the quality of the antibody preparation. Obtaining antibody solutions that are consistent in their concentration levels is currently a challenge for manufacturers of detection equipment.

Manufacturers will have their equipment tested in the field for ease of use and at various government sites using actual BT agents. Validation data are also required to substantiate their reliability claims. This report summarizes many of the commercially available systems; however their reliability in the field under daily use has not been proven. Detailed information can be obtained from the respective websites or contacting the manufacturers directly.

Detection Methods in Testing Phase

• Lawrence Livermore National Laboratories is working on a sandwich fluoroimmunoassay using beads (**Luminex Technology**) that are analyzed by flow cytometry. Different antibodies on each bead will allow for multiplex detection and thus hundreds of different pathogens will be detected in a single assay. Each bead is identified with a color code read by an optical bar code.

• Researchers at Argonne National Laboratory (ANL) have developed a special **biochip** platform that will provide a rapid, sensitive, reliable, and low-cost method for the detection of anthrax and other biological agents. The nucleic acid of the unknown organism is loaded onto a microarray of diagnostic oligonucleotides pre-positioned on a glass slide (the biochip) and subsequently measured by the degree of hybridization. The biochip reader and portable computer are currently undergoing evaluation.

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• Similarly, Evident Technologies is developing the use of nanocrystals for microarray analysis, commercialized in a product it calls the **EviArray**, which consists of EviDots (tiny crystals of cadmium selenide which fluoresce when hit with light) of many colors attached to specifically designed oligo sequences and anchored on a substrate to form a pre-labeled, oligo probe array. If the DNA in the unknown sample form complimentary base pairs with the probe, the tagged DNA moves which generates the fluorescence emission.

• Biral (UK) is working on **fluorescence-*ASAS*** *(***A**erosol **S**ize **A**nd **S**hape). Airborne particle populations are optically analyzed in real-time using laser scattering. The ambient aerosol is physically characterized in terms of size, shape and concentration to generate its characteristic signature. Since biological agents normally have their own representative size and shape, they are distinct from general background particles. Then neural network software monitors the aerosol background for certain systematic changes which would indicate an event.

• University of Southern California (USC) in Los Angeles, the University of California in Berkeley (UCB), and Oak Ridge National Laboratory (ORNL) researched and developed the **microcantilever technology**. UCB researchers made the cantilevers from silicon nitride using techniques identical to those used to make computer microprocessors. The cantilever's top surfaces are coated with antibodies to detect specific proteins or single-stranded DNA sequences to detect nucleotides. The cantilever bends when proteins bind to the antibodies or a DNA strand binds with a complementary DNA strand. The higher the protein or DNA concentration that sticks to the cantilever, the greater the deflection is. The deflection is then measured with a laser.

Commercially Available Systems

Bio-Seeq (Smiths Detection, Baltimore) is similar to Lawrence Livermore National Laboratories' HANAA, (Handheld Advanced Nucleic Acid Analyzer) which is a rapid DNA detection unit that utilizes polymerase chain reaction (PCR) technology in a compact handheld unit. Bio-Seeq can detect 1 CFU in 20 minutes. The FDA will be testing the HANAA to check imported seafood.

Cyflow (Partec, Germany) a DNA detection technique that works by placing suspect cells into a solution and directing a laser at the sample. The laser light scatters uniquely and an optical detector compares the pattern with the DNA of a known pathogen. This is similar to Lawrence Livermore's MiniFlo, which in field tests had a detection rate of 87 percent, with 0.5 percent false positives.

Smart Cycler (Cepheid, Sunnyvale, CA) enables rapid amplification and DNA detection to be carried out in a single reaction tube. It is also designed to carry out preparation and processing of raw specimens and reagents in a rapid, automatic, hands-off manner. The systems are designed as modules, to be integrated into a wide range of system configurations for rapid automatic DNA based analyses in a wide range of settings, including field testing.

RAPID or Ruggedized Advanced Pathogen Identification Device (Idaho Technology, Salt Lake City, UT) is capable of automatically analyzing samples for the presence of any DNA sequence. This instrument integrates LightCycler® Instrument technology into a portable, impact resistant package. Software allows the RAPID to automatically collect the data, interpret the test data, and report the results in less than 30 minutes.

APDS-II or Autonomous Pathogen Detection System was developed at Lawrence Livermore and is a fully automated stationary pathogen detector unit. It incorporates an aerosol collector, a sample preparation subsystem, and both PCR and flow cytometry detection capabilities to reduce false positives. Eventually APDS may be found at large meeting places such as airports and stadiums.

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The BioCapture™ BT-550 (Tetracore, Gaithersburg, MD) is a portable, bioaerosol collection and detection system that collects airborne pathogens and spores 0.5 microns and larger into a small volume of liquid.

BioGuardian (Innovateck, Richland, WA) is a high volume air sampling system that can collect and concentrate invisible airborne particles & chemicals. It uses a novel wet-walled multi-cyclone inner collector and has continuous or intermittent sampling capability. This would be used in conjunction with a detection device.

Bio-Detector (Smiths Detection, Baltimore) was jointly developed and tested with the U.S. Army Chemical and Biological Defense Command, and is an automated biological agent detector that simultaneously detects up to eight different agents using Immuno-ligand Assay chemistries. The test results are obtained in 15 minutes or less. Test samples are mixed with biotin and fluorescein-labeled antibody solutions as well as streptavidin. These are bathed in a solution of the substrate urea. The urease chemically reacts with the urea solution and causes a rapid change in pH. The rate of change is directly proportional to the amount of biological agent. The light-addressable potentiometric sensor (LAPS) measures the pH change and transmits an electrical signal to the signal processor. A pattern-recognition algorithm in the signal processor determines the presence of biological agents.

Smart-II (New Horizons Diagnostics, Columbia, MD) detects BT agents in environmental samples. A BT unknown is placed into a buffer that is squirted onto a paper strip laced with antibodies that will react with organisms. The appearance of a line denotes a positive test while the absence of a line is considered negative.

Raptor (Research International, Monroe, WA) uses optical biosensors, sandwich fluoroimmunoassays and evanescent wave technology to provide real-time detection with non-homogeneous samples. It is a multi-analyte detector with quantification capabilities. The Raptor has four optical fibers that can detect up to 8 analytes. It is fully automated using 10 minute assays and push button operation. Anthrax can be detected at 50 CFU/mL levels.

ANP Handheld Assay (ANP Technologies, Aberdeen, MD) places the fluorescent tag on the slide first followed by the binding of the antibody (of choice) which helps orientate the antibody during binding (and thus reduces randomness). This increases the sensitivity of the traditional antibody assay. Data is obtained within three minutes. Detection levels of *Bacillus anthracis* are at levels of 1000 cfu/mL. Incorporating microarray technology, currently underway, will provide for simultaneous detection of multiple biological agents.

The RAMP Anthrax Test (Meditech International, Huntington Beach, CA) is an immunoassay for the detection of *Bacillus anthracis*. The RAMP consists of an analyte-specific immunochromatographic strip (latex particles fluorescently labeled and tagged with antibodies) and a portable fluorescence reader that is used to quantify antibody-antigen complexes. The result is displayed in less than 15 minutes.

BioThreat Alert™ (Tetracore, Gaithersburg, MD) test strips employ a technology similar to that in use in many laboratory medical screening tests. Unlike embedded flow immunochromatography, where layers of chemical reagents are stacked upon each other, in lateral flow devices the chemical reagents are separated across the test strip. This lateral design provides for fewer false positives in environmentally collected samples. When the target substance (such as anthrax) is present at or above a detectable amount in the test sample, a visual reddish “band” will appear in the on the test strip. The band is an indication of an antibody-antigen complex above a certain concentration.

FDA Recalls Tests, continued from page 1

Roche Diagnostics' U.S. CoaguChek Web site (http://www.coaguchek-usa.com). Full text of FDA's recall notice can be found at the following website: http://www.fda.gov/cdrh/oivd/news.html.

VIDAS Chlamydia Assay: The Food and Drug Administration (FDA) Center for Devices and Radiological Health posted a Class I recall notice for the VIDAS Chlamydia Assay used in the laboratory on specimens collected from either symptomatic or asymptomatic patients for the qualitative detection of Chlamydia infections. The recalling firm is bioMerieux, Durham, N.C. A raw material, bovine serum albumin, contained in the VIDAS reagent strip, is causing an accelerated degradation of the product's performance and creating the potential for false negative results. Continued use of the defective assay could result in a moderate to high risk of serious adverse health consequences or death. Read the MedWatch 2003 safety summary, including links to the CDRH recall posting, at: http://www.fda.gov/medwatch/SAFETY/2003/safety03.htm#vidas.

Occupational Exposure to Bloodborne Pathogens

The Washington State Department of Labor and Industries (DLI) has rewritten and reorganized for clarity and ease of use the safety standards for occupational exposure to bloodborne pathogens. A copy of the rewritten Bloodborne Pathogen rules (Chapter 296-823 WAC) can be obtained at the following website: http://www.lni.wa.gov/wisha/rules/bbpathogens/default.htm, or by contacting your nearest DLI office.

E-mail Address and Fax Numbers Needed

The Medical Test Site (MTS) Licensing Program database is used for notification of physicians and laboratories of public health issues of immediate concern. In order to reach your facility in a more timely manner, we need to have the fax number and e-mail address, if available, for the person listed as the "laboratory contact" for your facility. Please send this information to vicky.terry@doh.wa.gov or fax to Vicky Terry at (206) 361-2813.

Still Time to Register!

10th Annual Clinical Laboratory Conference
November 10, 2003
Seattle Marriott Sea-Tac Hotel

Contact Leonard Kargacin at (206) 361-2804 or leonard.kargacin@doh.wa.gov for registration information.

Helpful Hints

Medical Test Sites (MTS) are required to notify the Laboratory Quality Assurance Office (LQA) within 30 days when:

√ tests are added or removed from the test menu for their facility.

√ there is a change in the owner, director, or laboratory contact for the facility.

New forms have been added to the LQA website to facilitate this process. They can be found at: www.doh.wa.gov/lqa.htm. Select the "Updates" tab on the left side of the screen.

√ Use the "Test Menu Change Notification Form" to submit information about added or deleted tests.

√ Use the "Personnel Change Notification Form" to submit changes about the owner, director, or laboratory contact for the facility.

Calendar of Events

PHL Training Classes:
(http://www.doh.wa.gov/EHSPHL/PHL/train.htm)

Shipping & Handling Biohazardous Materials

October 30	Shoreline
December 3	Moses Lake

Northwest Medical Laboratory Symposium

October 22-25	Olympia

10th Annual Clinical Laboratory Conference

November 10	Seattle

WSSCLS/NWSSAMT Spring Meeting

April 29 - May 1, 2004	Vancouver

Contact information for the events listed above can be found on page 2. The Calendar of Events is a list of upcoming conferences, deadlines, and other dates of interest to the clinical laboratory community. If you have events that you would like to have included, please mail them to ELABORATIONS at the address on page 2. Information must be received at least one month before the scheduled event. The editor reserves the right to make final decisions on inclusion.

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